Safety and efficacy of a three-dose regimen of *Plasmodium falciparum* sporozoite vaccine in adults during an intense malaria transmission season in Mali: a randomised, controlled phase 1 trial

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Summary

Background WHO recently approved a partially effective vaccine that reduces clinical malaria in children, but increased vaccine activity is required to pursue malaria elimination. A phase 1 clinical trial was done in Mali, west Africa, to assess the safety, immunogenicity, and protective efficacy of a three-dose regimen of *Plasmodium falciparum* sporozoite (PfSPZ) Vaccine (a metabolically active, non-replicating, whole malaria sporozoite vaccine) against homologous controlled human malaria infection (CHMI) and natural *P falciparum* infection.

Methods We recruited healthy non-pregnant adults aged 18–50 years in Donéguébougou, Mali, and surrounding villages (Banambani, Toubana, Torodo, Sirababougou, Zorokoro) for an open-label, dose-escalation pilot study and, thereafter, a randomised, double-blind, placebo-controlled main trial. Pilot study participants were enrolled on an as-available basis to one group of CHMI infectivity controls and three staggered vaccine groups receiving: one dose of $4·5\times10^5$, one dose of 9×10^5, or three doses of $1·8\times10^6$ PfSPZ via direct venous inoculation at approximately 8 week intervals, followed by homologous CHMI 5 weeks later with infectious PfSPZ by direct venous inoculation (PfSPZ Challenge). Main cohort participants were stratified by village and randomly assigned (1:1) to receive three doses of $1·8\times10^6$ PfSPZ or normal saline at 1, 13, and 19 week intervals using permuted block design by the study statistician. The primary outcome was safety and tolerability of at least one vaccine dose; the secondary outcome was vaccine efficacy against homologous PfSPZ CHMI (pilot study) or against naturally transmitted *P falciparum* infection (main study) measured by thick blood smear. Combined artesunate and amodiaquine was administered to eliminate pre-existing parasitaemia. Outcomes were analysed by modified intention to treat (mITT; including all participants who received at least one dose of investigational product; safety and vaccine efficacy) and per protocol (vaccine efficacy). This trial is registered with ClinicalTrials.gov, number NCT02627456.

Findings Between Dec 20, 2015, and April 30, 2016, we enrolled 56 participants into the pilot study (five received the $4·5\times10^5$ dose, five received 9×10^5, 30 received $1·8\times10^6$, 15 were CHMI controls, and one withdrew before vaccination) and 120 participants into the main study cohort with 60 participants assigned PfSPZ Vaccine and 60 placebo in the main study. Adverse events and laboratory abnormalities post-vaccination in all dosing groups were few, mainly mild, and did not differ significantly between vaccine groups (all p>0·05). Unexpected severe transaminitis occured in four participants: one participant in pilot phase that received $1·8\times10^6$ PfSPZ Vaccine, one participant in main phase that received $1·8\times10^6$ PfSPZ Vaccine, and two participants in the main phase placebo group. During PfSPZ CHMI, approximately 5 weeks after the third dose of $1·8\times10^6$ PfSPZ, none of 29 vaccinees and one of 15 controls became positive on thick blood smear; subsequent post-hoc PCR analysis for submicroscopic blood stage infections detected *P falciparum* parasites in none of the 29 vaccine recipients and eight of 15 controls during CHMI. In the main trial, 32 (58%) of 55 vaccine recipients and 42 (78%) of 54 controls became positive on thick blood smear during 24-week surveillance after vaccination. Vaccine efficacy (1–hazard ratio) was 0·51 per protocol (95% CI 0·20–0·70; log-rank p=0·0042) and 0·39 by mITT (0·04–0·62; p=0·033); vaccine efficacy (1–risk ratio) was 0·24 per-protocol (0·02–0·41; p=0·031) and 0·22 mITT (0·01–0·39; p=0·041).

Interpretation A three-dose regimen of PfSPZ Vaccine was safe, well tolerated, and conferred 51% vaccine efficacy against intense natural *P falciparum* transmission, similar to 52% vaccine efficacy reported for a five-dose regimen in a previous trial.

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For the French translation of the abstract see Online for appendix 1

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Research in context

Evidence before this study

We searched PubMed, the Cochrane Library, and other relevant data sources on Feb 12, 2021, for English-language articles on randomised controlled trials of malaria vaccines in adults published between Jan 1, 1980, and Feb 12, 2021. We searched using the following terms ("malaria vaccines" [MeSH Terms] OR "malaria" [All Fields] AND "vaccines" [All Fields]) OR "malaria vaccines" [All Fields] OR ("malaria" [All Fields] AND "vaccine" [All Fields]) OR "malaria vaccine" [All Fields]) AND (PfSPZ [All Fields] AND PfSPZ Vaccine [All Fields])) AND ("adults" [MeSH Terms] OR "adults" [All Fields]). For the Cochrane Library and other data sources, we used the key search terms "PfSPZ", "malaria vaccines", "adults", AND "clinical trials". Although *Plasmodium falciparum* sporozoite (PfSPZ) Vaccine studies have been previously conducted in malaria-endemic regions, only one trial of a whole malaria sporozoite vaccine in the field has reported efficacy against natural infection, using a five-dose PfSPZ Vaccine regimen. The partially effective RTS,S/AS01E vaccine was recently recommended by WHO for use in young children, but has not shown significant efficacy in adults.

Added value of this study

Our results build on an earlier field trial with promising protective efficacy results, wherein $2{\cdot}7\times10^5$ PfSPZ Vaccine given at 0, 28, 56, 84, and 140 days conferred significant protection to Malian adults against naturally occurring *P falciparum* infection across a transmission season. Here, three monthly doses of $1{\cdot}8\times10^6$ PfSPZ Vaccine were well tolerated and safe. There were no significant differences in local or systemic reactogenicity nor in laboratory abnormalities between PfSPZ Vaccine and placebo recipients nor any related serious events reported. 78% of our adult controls developed *P falciparum* infection. Vaccine efficacy was 51% by time to infection analysis (95% CI 20–70%; log-rank p=0·0042), and 24% by proportional analysis (2–41; p=0·031) per-protocol. *P falciparum* circumsporozoite protein antibody response and Vδ2 γδ T cell increase after vaccination were significantly related to infection risk during follow-up.

Implications of all the available evidence

Findings from our study confirm the protective efficacy of PfSPZ Vaccine against naturally occurring *P falciparum* infection in Mali, and establish an efficacious three-dose regimen that is safe, well tolerated, and practical for implementation.

Introduction

WHO reported 229 million malaria cases and 409 000 deaths in 2019, with no meaningful reductions in case numbers since 2013 or deaths since 2016.[1] A highly effective vaccine is urgently needed to stem resurgences, break the stalemate in progress, and ultimately eliminate *Plasmodium falciparum* from highly endemic areas. The RTSS/AS01E vaccine recently recommended by WHO has partial efficacy to reduce clinical malaria in children,[2] but has not shown significant efficacy in adults.[3]

P falciparum sporozoite (PfSPZ) Vaccine (Sanaria, Rockville, MD, USA) is a malaria vaccine candidate, consisting of aseptic, purified, cryopreserved whole malaria sporozoites that are metabolically active, motile, able to invade hepatocytes, but non-replicating and unable to progress to blood-stage infection. Attenuated sporozoites are thought to protect by eliciting CD8 T-cell responses targeting infected hepatocytes.[4] Preventing blood-stage infection will prevent disease as well as onward transmission to support malaria elimination.

See Online for appendix 2

In our previous trial of PfSPZ Vaccine in Mali where *P falciparum* transmission is seasonally intense, five doses of $2{\cdot}7\times10^5$ PfSPZ achieved vaccine efficacy of 52% by time to infection (1–hazard ratio) over the 24-week transmission season.[5] However, three doses or fewer would facilitate vaccine implementation, particularly in mass vaccination programmes for malaria elimination.[6] In this trial, in the same village, we assessed three doses of $1{\cdot}8\times10^6$ PfSPZ, increasing total dose four-fold from $1{\cdot}35\times10^6$ to $5{\cdot}4\times10^6$ PfSPZ. The primary objective assessed safety and tolerability and the secondary assessed vaccine efficacy that prevented blood stage infection during homologous PfSPZ CHMI (pilot) or due to naturally transmitted P *falciparum* (main) infection in healthy Malian adults.

Methods

Study design and participants

We did a two-part trial: first, an open-label pilot study of safety of two dose escalations of PfSPZ Vaccine ($4{\cdot}5\times10^5$ to 9×10^5 to $1{\cdot}8\times10^6$ PfSPZ) and vaccine efficacy of $1{\cdot}8\times10^6$ PfSPZ against homologous (NF54) controlled human malaria infection (CHMI); then, a randomised, double-blind, placebo-controlled safety and efficacy trial ($1{\cdot}8\times10^6$ PfSPZ *vs* normal saline), where participants were followed up for incident malaria infections by thick blood smear during the ensuing rainy season (appendix 2 p 19). The trial involved a single centre in Donéguébougou, Mali, a rural community about 30 km north of Bamako, Mali. Malaria transmission usually occurs between July and December.[7]

Eligible participants were healthy adult (18–50 years) non-pregnant women who used contraception during the vaccination phase or men who resided in Donéguébougou, Mali, and surrounding villages (Banambani, Toubana, Torodo, Sirababougou, Zorokoro). Each participating village provided community permission; all participants provided individual written informed consent.[8] Exclusion criteria included known allergies or contraindications to PfSPZ Vaccine or combined artesunate and amodiaquine,

malaria vaccine within 5 years, abnormal laboratory findings, recent antimalarial medications, immunosuppressive medications, or blood products, a history of serious chronic illness, clinically significant electrocardiogram abnormalities, positive test for HIV, hepatitis B, hepatitis C, or known sickle cell disease (full list of inclusion and exclusion criteria is available in appendix 2 [pp 6–8]).

The trial was done according to Good Clinical Practice and International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use guidelines and institutional procedures and guidelines. The study was approved by the ethics review board in Mali (Faculté de Médecine de Pharmacie et d'Odonto Stomatologie, Bamako, Mali), the US National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH; Bethesda, MD, USA) institutional review board, and the Mali national regulatory authority. NIAID was the study clinical sponsor and Sanaria was the Investigational New Drug sponsor, under a US Food and Drug Administration (FDA) Investigational New Drug allowance.

Randomisation and masking

For safety, we conducted the trial in a stepwise manner with two cohorts: the pilot dose-escalation open-label safety and CHMI cohort, and the follow-on main cohort. Pilot dose-escalation and CHMI participants were enrolled on an as-available basis. Main cohort participants were stratified by village (with a total of six villages) and randomly assigned in double-blind manner using permuted block design. Participants were assigned (1:1) to three doses of $1·8\times10^6$ PfSPZ Vaccine or normal saline placebo. The randomisation code was provided directly by the study statistician to the site pharmacist via secure email before vaccinations started. Investigational product was labelled by the pharmacist with participant's study identification number. PfSPZ Vaccine and placebo were clear, odourless, non-viscous solutions and could not be distinguished. Group assignments were unmasked at final study visit 24 weeks after the third vaccination.

Procedures

Sanaria PfSPZ Vaccine (Rockville, MD, USA) contains aseptic, purified, vialed, cryopreserved PfSPZ manufactured as described previously.[4,9,10] Within 30 min of thawing, 0·5 mL of PfSPZ Vaccine or Sanaria PfSPZ Challenge[11–13] or placebo (sterile isotonic normal saline [Hospira, Lake Forest, IL, USA]) was injected into an arm vein by direct venous inoculation through 25-gauge needle over several seconds.

For safety, the pilot study enrolled in a staggered manner the three PfSPZ Vaccine groups: one dose of $4·5\times10^5$ (January, 2016), one dose of 9×10^5 (January, 2016), and three doses of $1·8\times10^6$ (January–May, 2016) at approximately 8-week intervals. The group receiving $1·8\times10^6$ PfSPZ Vaccine was randomly assigned (1:1) before first vaccination to receive artesunate and amodiaquine before each vaccine dose or only before the third dose (and thus before CHMI) alongside CHMI infectivity controls.

The pilot group receiving $1·8\times10^6$ PfSPZ Vaccine underwent homologous CHMI approximately 5 weeks after the third dose (June, 2016). CHMI infectivity controls were enrolled in April, 2016, and as noted above, both PfSPZ Vaccine and controls were treated with artesunate and amodiaquine approximately 7 weeks before CHMI. All CHMI participants were followed up for patent parasitaemia 4 weeks post-challenge and treated with combined artemether and lumefantrine for parasitaemia or at end of follow-up. The $1·8\times10^6$ PfSPZ pilot cohort received a fourth dose (13 weeks after dose three) contemporaneous with main cohort dose three.

The main trial (March–August, 2016) randomly allocated participants to three doses of $1·8\times10^6$ PfSPZ or normal saline placebo at 1, 13, and 19 week intervals.

After each vaccination, participants were monitored for at least 30 min for local and systemic adverse events. Participants were assessed on-site immediately and then 3, 7, 14, 28, 42, and 56 days post-vaccination, and study clinicians were always available for unscheduled visits. Solicited local and systemic adverse events were recorded for 7 days post-vaccination (appendix 2 p 21). Unsolicited adverse events, including symptomatic malaria, serious adverse events, and new chronic conditions were recorded throughout follow-up. Protocol-specified laboratory assessments before vaccination and 3 and 7 days after each vaccination included complete blood count with differential, creatinine, and alanine aminotransferase. Adverse event grading was based on FDA guidelines for vaccine trials[14] adapted to local normal reference ranges (appendix 2 pp 22–23).

We deemed participants to be enrolled upon artesunate and amodiaquine (Denk Pharma; Munich, Germany) treatment 1–2 weeks before first vaccination. Two tablets (combined 100 mg artesunate and 270 mg amodiaquine each) were given twice daily for 3 days (six doses total). All pilot $1·8\times10^6$ PfSPZ Vaccine recipients and main trial participants received artesunate and amodiaquine again approximately 2 weeks before third vaccination.

We assessed co-infections before first vaccination. Gastrointestinal helminths or protozoa were detected in stool by modified qPCR[15] at the Laboratory of Parasitic Diseases, NIAID/NIH. Helminth and protozoa screening included testing for *Ascaris lumbricoides, Necator americanus, Ancylostoma duodenale, Giardia lamblia, Cryptosporidium* spp, *Entamoeba histolytica, Trichuris trichuria,* and *Strongyloides stercoralis*. *Schistosoma haematobium* eggs were quantified microscopically in fresh urine post-filtration and staining with 5% ninhydrin at the College of American Pathologists-certified Malaria Research and Training Centre (MRTC) clinical laboratory.[5,15]

Thick blood smears were prepared before and at prespecified visits after each vaccination, and during

suspected malaria illness (appendix 2 pp 9–10). Thick blood smears were examined by certified readers. Symptomatic malaria was defined as any *P falciparum* asexual parasitaemia accompanied by temperature of 37·5°C or higher, clinical signs or symptoms of malaria, or both. Standard treatment with combined artemether and lumefantrine was provided for symptomatic malaria, but not asymptomatic parasitaemia per Malian Ministry of Health guidelines (except during CHMI).

CHMI started 5 weeks after dose three by inoculation of $3·2\times10^3$ of non-attenuated PfSPZ Challenge (NF54), the same parasite strain as PfSPZ Vaccine. Thick blood smears were collected on day 3, daily on days 6–21, every other day on days 23–27, and when clinically indicated. Paired qPCR samples were collected with each thick blood smear but assayed retrospectively.

For main trial follow-up, thick blood smears began 3, 7, and 14 days after third vaccination, then continued every 2 weeks for 11 additional scheduled assessments, and when clinically indicated, ending after 24 weeks.

Serum antibodies were measured by ELISA to the major sporozoite surface protein (*P falciparum* circumsporozoite protein [PfCSP]), by automated immunofluorescence assay to air-dried PfSPZ, and by automated inhibition of sporozoite (PfSPZ) invasion assay of HC-04 cells (hepatocytes) as previously described.[16] PfCSP ELISA seroconversion was defined by net optical density 1·0 and optical density 1·0 ratio, calculated by subtracting or dividing by the pre-vaccination antibody optical density 1·0, of 50 or greater and 3·0 or greater, respectively.[16] For automated immunofluorescence assay, volunteers with net arbitrary fluorescence unit (AFU) $2·0\times10^5$ of 150 or greater and a ratio of post-vaccination to pre-vaccination AFU $2·0\times10^5$ of 3·0 or greater were considered to have responded positively. In the automated inhibition of sporozoite invasion assay, volunteers with a net inhibition of sporozoite invasion activity of 10% or greater and ratio of post-vaccination to pre-vaccination inhibition of sporozoite invasion activity of 3·0 or greater were considered to have developed inhibition of sporozoite invasion activity.

We assessed T-cell responses using multiparameter flow cytometry on fresh whole blood (appendix 2 p 10, 47). Ex-vivo measures were taken before vaccination and at 3, 7, 42, and 55 days after each vaccination, and every 4 weeks during follow-up.

Outcomes

The primary outcome was safety and tolerability of at least one vaccine dose, a modified intention-to-treat (mITT) analysis assessed as incidence and severity of local and systemic adverse events occurring within 7 days of each vaccination and severe adverse events related to vaccination. Planned secondary outcomes were vaccine efficacy against blood stage infection during CHMI in the pilot study measured by thick blood smear, and in the main trial vaccine efficacy by time-to-infection analysis (1–hazard ratio) and by binary analysis (1–risk ratio) against naturally occurring *P falciparum* infection by thick blood smear (defined as at least two parasites identified by microscopic examination of 0·5 µL blood). Vaccine efficacy against symptomatic malaria and CHMI vaccine efficacy by qPCR were exploratory outcomes. CHMI vaccine efficacy was defined as 1–(proportion of infection under vaccine/proportion of infection under control). Humoral and cellular immune responses (appendix 2 p 10) were exploratory endpoints. Planned outcomes were not changed during the trial, except for additional analysis of CHMI outcomes detailed here.

Statistical analysis

All participants who received at least one dose of investigational product (PfSPZ Vaccine or placebo) were included in safety analyses, including the pilot safety cohort.

For the pilot study, the prespecified secondary outcome was time to first blood stage infection measured by thick blood smear during CHMI, but as only one infectivity control was thick blood smear positive, time to first blood stage infection measured by qPCR was also analysed with R version 3.3.1.

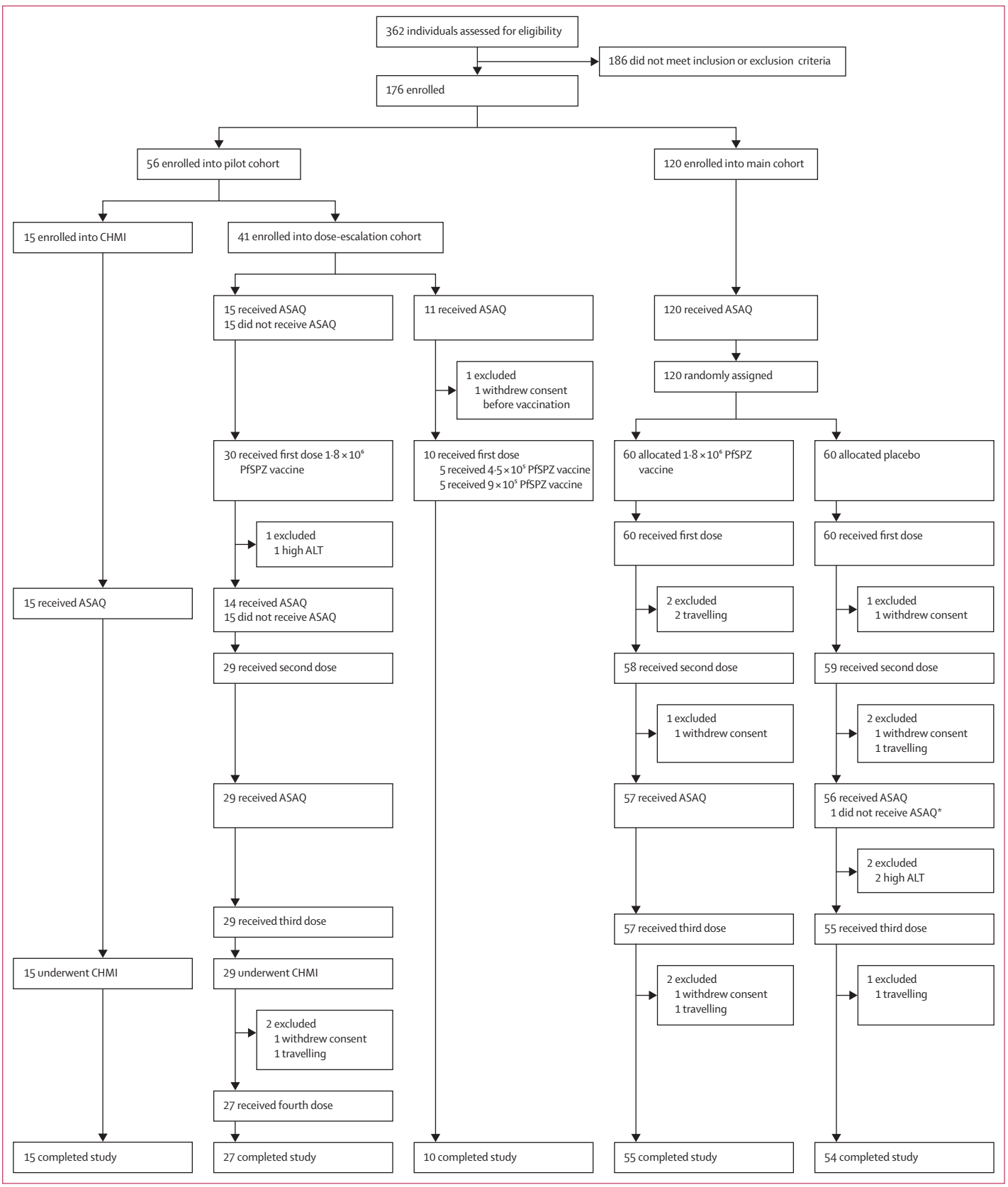
For the main cohort, 60 participants per group (PfSPZ Vaccine and placebo) provided 0·8 probability of observing serious or severe adverse events that occurred with probability of 0·026 per volunteer. With background malaria infection rate varying from 40% to 90%, we expected to detect a time to infection vaccine efficacy of at least 50% with 59–100% power (two-sided 0·05 conditional test; appendix 2 pp 32–33).

All randomly assigned main cohort participants were included or accounted for in the mITT analysis whereas only those who received all three vaccinations were included in the per-protocol analysis. The main efficacy endpoint was time to first infection, with vaccine efficacy defined as 1–hazard ratio. Imputation for missed visits for the mITT and per-protocol analyses is described in more detail in the appendix 2 (p 12).

Vaccine efficacy was assessed two ways: (1) time-to-event analysis, in which significance was assessed by log-rank test for interval-censored data, and vaccine efficacy was evaluated from a parametric proportional hazards model assuming baseline hazard function as

Figure 1: **Trial profile**

Study completion for pilot $4·5\times10^5$, 9×10^5 PfSPZ Vaccine recipients was defined as staying to the end of safety follow-up post-vaccination; for CHMI infectivity controls, to the end of the CHMI period; for pilot and main $1·8\times10^6$ PfSPZ Vaccine recipients, to the end of the malaria transmission follow-up (study day 85 for $4·5\times10^5$ or 9×10^5 PfSPZ Vaccine; study day 30 for CHMI controls; study day 357 for pilot $1·8\times10^6$ PfSPZ Vaccine; study day 281 for main cohort $1·8\times10^6$ PfSPZ Vaccine and placebo). CHMI=controlled human malaria infection. ASAQ=combined artesunate and amodiaquine. PfSPZ=*Plasmodium falciparum* sporozoite. ALT=alanine transaminase. *One normal saline participant did not receive ASAQ dose between vaccine doses two and three because they were recently treated for malaria with artemether–lumefantrine.


362 individuals assessed for eligibility
186 did not meet inclusion or exclusion criteria
176 enrolled
56 enrolled into pilot cohort
120 enrolled into main cohort
15 enrolled into CHMI
41 enrolled into dose-escalation cohort
15 received ASAQ
15 did not receive ASAQ
11 received ASAQ
120 received ASAQ
1 excluded
1 withdrew consent before vaccination
120 randomly assigned
30 received first dose 1·8 × 10⁶ PfSPZ vaccine
10 received first dose
5 received 4·5 × 10⁵ PfSPZ vaccine
5 received 9 × 10⁵ PfSPZ vaccine
60 allocated 1·8 × 10⁶ PfSPZ vaccine
60 allocated placebo
1 excluded
1 high ALT
60 received first dose
60 received first dose
15 received ASAQ
14 received ASAQ
15 did not receive ASAQ
2 excluded
2 travelling
1 excluded
1 withdrew consent
29 received second dose
58 received second dose
59 received second dose
1 excluded
1 withdrew consent
2 excluded
1 withdrew consent
1 travelling
29 received ASAQ
57 received ASAQ
56 received ASAQ
1 did not receive ASAQ*
2 excluded
2 high ALT
29 received third dose
57 received third dose
55 received third dose
15 underwent CHMI
29 underwent CHMI
2 excluded
1 withdrew consent
1 travelling
2 excluded
1 withdrew consent
1 travelling
1 excluded
1 travelling
27 received fourth dose
15 completed study
27 completed study
10 completed study
55 completed study
54 completed study

Weibull and allowing for interval censoring and stratification by village; and (2) proportion of participants with at least one positive blood smear (binary endpoint) in each group by exact Cochran-Mantel-Haenszel test stratifying on village.

Participants whose data were censored before 160 days after last vaccination were removed from the proportional analysis on efficacy, as prespecified in the protocol.

For PfCSP antibody measurements, we analysed differences between vaccinees and controls, or between uninfected and infected vaccinees, using two-tailed Barnard's test or Fisher's exact test for seroconversion rates, and Wilcoxon rank-sum test for net optical density and optical density ratios. We analysed changes in T-cell levels between the PfSPZ and placebo groups and by infection outcome within groups. T-cell levels or fold changes were compared between groups at specific timepoints using the Wilcoxon rank-sum test. Repeated measures analysis was done by fitting linear models using generalised estimating equations (GEE) to assess differences between using $\log_{10}$ transformed fold change values as the outcome variable. For comparisons between vaccine groups study group, day, and an interaction term, study group×day, were used as the predictor variables. For comparisons between infected and uninfected participants within vaccine groups, study day and infection outcome were used as the predictor variables. Age and sex of study participants were used as confounding variables in the full models, neither of which were significant. A Gaussian (normal) distribution, identity link function, and an autocorrelation covariance structure were specified. A robust (sandwich) variance estimator was applied to all models (further details are available in the appendix 2 [pp 13–14]).

The study was monitored for safety by an independent data and safety monitoring board and a local medical monitor. This trial is registered at ClinicalTrials.gov, number NCT02627456.

Role of the funding source

The funders were involved in study design and management, data collection, analysis, and interpretation, and report writing.

Results

Participants (pilot and main) were screened and enrolled from Dec 20, 2015 to April 30, 2016. 56 individuals enrolled into the pilot cohort, one of whom withdrew before vaccination. 40 pilot dose-escalation participants received one dose of either $4·5×10^5$ (n=5) or $9×10^5$ (n=5) or three doses at 8-week intervals of $1·8×10^6$ (n=30) of PfSPZ Vaccine (before the start of main cohort; figure 1). 15 participants enrolled as CHMI controls received artesunate and amodiaquine, along with 29 participants receiving $1·8×10^6$ PfSPZ, 7 weeks before CHMI. 27 pilot study participants receiving $1·8×10^6$PfSPZ opted to receive a fourth dose (figure 1).

120 participants were enrolled into the main cohort and randomly assigned: 60 received PfSPZ Vaccine and 60 placebo. First main cohort vaccinations occurred April 1–8, 2016, last vaccinations Aug 4–13, 2016 (after onset of malaria transmission season), and final study visits Jan 19–26, 2017. Main cohort vaccinations were originally scheduled for weeks 1, 9, and 17 but were completed at weeks 1, 13, and 19, with second and third vaccinations delayed due to an investigation of an out-of-specification phosphate buffered saline stability test result that was later determined to be invalid allowing resumption of immunisation. All 120 participants received at least one vaccination and are included in the safety analyses. 112 participants (57 in the vaccine group and 55 in the placebo group) received all three vaccinations, of which 109 (55 in the vaccine group and 54 in the placebo group) completed follow-up through to last study visit (figure 1). Baseline characteristics were well balanced between vaccine and placebo groups (table 1).

Of the main cohort participants who received at least one vaccination, seven (12%) of 60 in the vaccine group and eight (13%) of 60 in the placebo group were positive on thick blood smear for *P falciparum* at screening (table 1). There were no significant differences between the groups with regard to helminths or protozoa in the stool, *S haematobium* in the urine, or haemoglobin AA, AS, or AC (table 1; appendix 2 p 51). All 120 participants completed artesunate and amodiaquine treatment before first vaccination, with the last dose given to the vaccine group a mean of 9·4 days (SD 1·1) and to the placebo group a mean of 9·4 days (SD 1·4) before first vaccination. During vaccination, one (2%) PfSPZ Vaccine receipient and three (5%) placebo participants developed parasitaemia, all occurring between the second and third vaccination. All 114 participants remaining in the main cohort received anti-malarial treatment (113 artesunate and amodiaquine by protocol, one artemether–lumefantrine due to malaria diagnosis) before their last vaccination, with the last drug dose given to the vaccine group a mean of 7·8 days (SD 0·5) and to the placebo group a mean of 7·8 days (0·8) before last vaccination.

For the pilot study, PfSPZ Vaccine was very well tolerated in those receiving $4·5×10^5$ (n=5) and $9×10^5$ (n=5) with few adverse events after vaccination and no grade 3 adverse events or laboratory abnormalities reported. $1·8×10^6$ PfSPZ vaccinations were also well tolerated by the majority of participants; however, one participant (randomly allocated to artesunate and amodiaquine treatment before each vaccination) experienced an asymptomatic grade 3 elevated alanine transaminase following first vaccination (appendix 2 pp 14, 29–30). Reported adverse events after CHMI were few, with only one related adverse event (granulocyte decreased), deemed related to CHMI. Safety summaries for the pilot and CHMI cohorts are provided in more detail in appendix 2 (pp 55–58).

For the main trial, 349 injections were administered and were well tolerated and safe. Most study participants reported no local or systemic adverse events after vaccination (table 2). Four (7%) of the 60 vaccine group participants and five (8%) of the 60 placebo group participants reported local injection site pain. Overall, three (5%) participants in the vaccine group and three (5%) participants in the placebo group reported any

	Pilot safety cohort plus CHMI (n=55)				Main cohort (n=120)		Total (n=175)
	PfSPZ Vaccine			CHMI infectivity controls (n=15)	PfSPZ Vaccine $1·8\times10^{6}$ (n=60)	Placebo (n=60)	
	$4·5\times10^{5}$ (n=5)	9×10^{5} (n=5)	$1·8\times10^{6}$ (n=30)				
Sex							
Female	3 (60%)	1 (20%)	11 (37%)	3 (20%)	15 (25%)	22 (37%)	55 (31%)
Male	2 (40%)	4 (80%)	19 (63%)	12 (80%)	45 (75%)	38 (63%)	120 (69%)
Age, years*							
Mean (SD)	36 (9)	34 (10)	37 (9)	30 (10)	32 (9)	32 (10)	33 (9)
Range	23–45	22–50	18–50	18–50	8–49	18–48	18–49
Weight, kg†							
Mean (SD)	54 (2)	53 (2)	61 (8)	61 (11)	62 (9)	62 (9)	61 (9)
Range	53–58	51–55	51–77	46–80	46–99	46–93	46–99
Village							
Doneguebougou	5 (100%)	5 (100%)	30 (100%)	10 (67%)	27 (45%)	28 (47%)	105 (60%)
Toubana	0	0	0	2 (13%)	12 (20%)	13 (22%)	27 (15%)
Banambani	0	0	0	2 (13%)	8 (13%)	8 (13%)	18 (10%)
Torodo	0	0	0	0	7 (12%)	6 (10%)	13 (7%)
Zorokoro	0	0	0	1 (7%)	3 (5%)	2 (3%)	6 (3%)
Sirababougou	0	0	0	0	3 (5%)	3 (5%)	6 (3%)
Haemoglobin typing‡							
Hgb AA	2 (40%)	5 (100%)	23 (77%)	7 (47%)	37 (62%)	39 (65%)	113 (65%)
Hgb AS	1 (20%)	0	2 (7%)	1 (7%)	3 (5%)	4 (7%)	11 (6%)
Hgb SS	0	0	0	0	0	0	0
Hgb AC	1 (20%)	0	4 (13%)	3 (20%)	11 (18%)	8 (13%)	27 (15%)
Hgb CC	1 (20%)	0	1 (3%)	0	0	0	2 (1%)
Unknown	0	0	0	4 (27%)	9 (15%)	9 (15%)	22 (13%)
***Schistosoma haematobium* (urine)§**							
Positive	0	0	2 (7%)	4 (27%)	7 (12%)	5 (8%)	18 (10%)
Helminth or parasite qPCR (stool)¶							
Positive	2 (40%)	4 (80%)	14 (47%)	6 (40%)	26 (43%)	30 (50%)	82 (47%)
Negative	3 (60%)	1 (20%)	15 (50%)	6 (40%)	32 (53%)	28 (47%)	85 (49%)
NIC or not done	0	0	1 (3%)	3 (20%)	2 (3%)	2 (3%)	8 (5%)
***Plasmodium falciparum* parasitaemia by blood smear‖**							
Pre-vaccination (n=160)	0	2 (40%)	7 (23%)	3 (20%)	7 (12%)	8 (13%)	27 (17%)
During vaccination (n=160)	0	0	6 (20%)**	NA	1 (2%)	3 (5%)	10 (6%)
Post vaccination one (n=160)	0	0	5 (17%)	NA	0	0	5 (3%)
Post vaccination two (n=146)	NA	NA	4 (14%)	NA	1 (2%)	3 (5%)	8 (6%)
Post vaccination three (n=138)	NA	NA	0	NA	32 (58%)	42 (78%)	74 (54%)
Post CHMI (n=44)	NA	NA	0	1 (7%)	NA	NA	1 (2%)
Post vaccination four (n=27)	NA	NA	18 (67%)	NA	NA	NA	18 (67%)

Data are n (%), unless stated otherwise. PfSPZ=*Plasmodium falciparum* sporozoite. CHMI=controlled human malaria infection. NIC=negative internal control, cannot rule out false negatives due to PCR inhibition. NA=not applicable. *Age is based on age at the time of enrollment (first dose of artesunate and amodiaquine). †Weight is based on weight measured at the time of screening. ‡Haemoglobin typing completed retrospectively. §Schistosomiasis testing completed at screening. ¶Helminth testing was completed retrospectively (appendix pp 50–52). ‖Post dose % are calculated based on n at that time interval as outlined in figure 1. Thick blood smear counts after dose three in the main cohort and after dose four in the pilot safety cohort is only for the 24 week period post-vaccination; per-protocol proportional population is included as the n. **All positive thick blood smears during vaccination in pilot $1·8\times10^{6}$ occurred in the arm randomly assigned to no artesunate and amodiaquine until dose three except for one participant who was positive after dose two.

Table 1: **Baseline demographics of participants who received at least one vaccination, pilot and main cohorts, and parasitaemia characteristics by thick blood smear**

	$1{\cdot}8\times10^6$ PfSPZ Vaccine				Placebo			
	Dose one (n=60)	Dose two (n=58)	Dose three (n=57)	Total (n=60)	Dose one (n=60)	Dose two (n=59)	Dose three (n=55)	Total (n=60)
Adverse events								
Local reactogenicity*	2 (2, 3%)	2 (2, 3%)	0	4 (4, 7%)	1 (1, 2%)	4 (4, 7%)	1 (1, 2%)	6 (5, 8%)
Systemic reactogenicity	1 (1, 2%)	5 (2, 3%)	1 (1, 2%)	7 (3, 5%)	3 (2, 3%)	1 (1, 2%)	0	4 (3, 5%)
Laboratory abnormalities†	1 (1, 2%)	5 (5, 9%)	4 (4, 7%)	10 (8, 13%)	1 (1, 2%)	2 (2, 3%)	2 (2, 4%)	5 (2, 3%)
Related adverse events‡	4 (4, 7%)	11 (7, 12%)	5 (5, 9%)	20 (12, 20%)	5 (4, 7%)	7 (6, 10%)	3 (2, 4%)	15 (9, 15%)
Unsolicited adverse events§	12 (9, 15%)	19 (15, 26%)	61 (32, 56%)	92 (42, 70%)	20 (18, 30%)	30 (19, 32%)	66 (37, 67%)	116 (50, 83%)
Serious adverse events	0	0	0	0	1 (1, 2%)	0	1 (1, 2%)	2 (2, 3%)
Symptomatic malaria adverse events¶								
Total	0	1 (1, 2%)	26 (24, 42%)	27 (25, 42%)	0	1 (1, 2%)	37 (31, 56%)	38 (31, 52%)
Grade 1	0	0	19 (18, 32%)	19 (18, 30%)	0	1 (1, 2%)	24 (20, 36%)	25 (20, 33%)
Grade 2	0	1 (1, 2%)	7 (7, 12%)	8 (8, 13%)	0	0	11 (11, 20%)	11 (11, 18%)
Grade 3	0	0	0	0	0	0	2 (2, 4%)	2 (2, 3%)
Grade 4	0	0	0	0	0	0	0	0

Data are number of adverse events (number of unique participants with events, % of unique participants). Each vaccine receipt is counted once at worst severity for any local and systemic parameter. Laboratory adverse events are shown in the appendix (pp 27–28). PfSPZ=*Plasmodium falciparum* sporozoite. *All local reactogenicity reported were injection site pain. †Laboratory abnormalities were included in this count if they occurred during the scheduled day 7 post-vaccination visit and were within window for that visit (up to day 9). ‡Related adverse events includes all adverse events reported within 28 days of vaccination and determined as definitely, probably, or possibly related to vaccination; includes expected reactogencity as well as laboratory abnormalities. §Unsolicited adverse events represented below does not include malaria adverse events, but does include laboratory adverse events occurring outside the predefined collection period post vaccination. All unsolicited adverse events were determined not related to vaccination except for two transaminases increased in the placebo arm (onset 33, 39 days after the second dose, determined possibly related during the study, grade 4 and grade 3; further details provided in the appendix [p 29]). ¶Although reported as malaria adverse events, 2 malaria adverse events are excluded for PfSPZ Vaccine given detection of *Plasmodium malariae* only, no *P falciparum,* and 1 malaria adverse event is excluded in the placebo given detection of *P malariae* and *Plasmodium ovale* only, no *P falciparum*.

Table 2: **Adverse events after vaccination in the main cohort (n=120)**

systemic adverse event after vaccination (table 2); the most common solicited systemic adverse event in the vaccine and placebo groups was headache (appendix 2 pp 25–26). Local or systemic adverse events did not differ significantly between vaccine and placebo groups (all p values >0·05; table 2; appendix 2 pp 25–26). Two serious adverse events were reported (snake bite, vaginal prolapse repair), both not related to vaccination and both occurring in placebo participants (table 2).

Of significance, during the pilot and main phase of the study, we noted multiple unanticipated substantially elevated, but asymptomatic, transaminases (grade 3, 4) in four participants (unexpected severe transaminitis occurred in one participant in pilot phase that received $1{\cdot}8\times10^6$ PfSPZ Vaccine, one participant in main phase that received $1{\cdot}8\times10^6$ PfSPZ Vaccine, and two participants in the main phase placebo group) that occurred at varying timepoints after vaccination as well as artesunate and amodiaquine dosing (appendix 2 pp 13–14, 27–28). All four participants were asymptomatic at presentation with no associated agranulocytosis. All laboratory abnormalities resolved without sequelae. Testing for potential other aetiologies, through imaging, expanded laboratory testing, and serology, identified no other possible contributing causes, except a traditional medicine ingested by the first participant who presented with liver enzyme derangements (appendix 2 pp 13–14). The cause of elevated transaminases was judged to be most likely due to artesunate and amodiaquine treatment given equal involvement of PfSPZ Vaccinees and controls.

Overall, laboratory abnormalities within 7 days after vaccination did not differ between the vaccine (eight [13%] of 60) and placebo (two [3%] of 60; p=0·095, Fisher's exact test) groups (table 2; appendix 2 pp 27–28). All laboratory abnormalities immediately after vaccination were grade 1 except for the transaminase elevations described here.

For the pilot study CHMI, none of 29 vaccinees and one (7%) of 15 infectivity controls became positive on thick blood smear. By qPCR, none of 29 vaccinees and eight (53%) of 15 infectivity controls became positive (appendix 2 p 61). By qPCR, vaccine efficacy was significant (p<0·0001) by interval-censored log rank for time to infection, and was 1·00 (p<0·0001, 95% CI 0·73–1·00) by proportional analysis. All 27 PfSPZ Vaccine recipients receiving a fourth dose were followed up for naturally occurring infection with the main trial cohort; 18 (67%) of 27 developed patent parasitaemia over the 24-week follow up.

For the main trial, vaccine efficacy analysis examined time to first *P falciparum* infection (1–hazard ratio) and occurrence of *P falciparum* infection (proportional [1–risk ratio]) during a 24-week period up to the end of the malaria season, starting immediately after the third vaccination (starting the week of Aug 4, 2016). *P falciparum* infection was defined as a positive thick blood smear. In the per-protocol population, follow up

time was a median of 167 and a mean of 162·5 (95% CI 156·0–169·0) days in the vaccine group, and a median of 167 and a mean of 164·5 (159·5–169·6) days in the control group. In the mITT population, follow up time was a median of 167 and a mean of 162·5 (156·0–169·0) days in the vaccine group, and a median of 167 and a mean of 164·6 (159·7–169·5) days in the control group.

Vaccine efficacy was assessed primarily from the per-protocol population, and efficacy is also reported for the mITT population. 112 participants (57 in the vaccine group and 55 in the placebo group) had evaluable data for time to first infection per-protocol analysis, while 114 participants (57 in the vaccine group and 57 in the placebo group) were evaluable for mITT analysis. PfSPZ Vaccine recipients had a significantly lower hazard of *P falciparum* infection, per protocol, with vaccine efficacy (1–hazard ratio) of 0·51 (95% CI 0·20–0·70; log-rank p=0·0042; figure 2). In mITT analyses, vaccine efficacy was 0·39 (0·04–0·62; log-rank p=0·033). We assessed the assumption of proportional hazards via Schoenfeld residuals, which indicated no violation of the proportional hazards assumption, with p values of 0·26 in the per-protocol and 0·23 in the mITT population (appendix 2 p 54).

In the proportional analysis of vaccine efficacy, 109 participants (55 in the vaccine group and 54 in the placebo group) were evaluable per protocol (figure 2), and 111 participants (55 in the vaccine group and 56 in the placebo group) by mITT (appendix 2 p 36). In the placebo group, 42 (78%) of 54 participants became blood smear positive versus 32 (58%) of 55 in the vaccine group. Vaccine efficacy (1–risk ratio) based on the binary outcome of infected or not during the season, using an exact Cochran-Mantel-Haenszel test stratified on village, was 0·24 (95% CI 0·02–0·41; p=0·031) per protocol, and 0·22 (0·01–0·39; log-rank p=0·041) by mITT analysis (appendix 2 p 36).

In a proportional analysis that included all participants, an empirical likelihood estimation was applied to the data (including censored participants) to estimate the proportion of infection by the end of the 24-week follow-up. For the per-protocol population, the proportion of infection was 0·76 (95% CI 0·64–0·86) in the control group, and 0·56 (0·43–0·69) in the vaccine group. The estimate of vaccine efficacy was 26%, slightly higher than the 24% based on proportional analysis that removed censored participants.

Among 112 main cohort participants who received all three vaccinations, 25 (42%) of 57 in the vaccine group and 31 (56%) of 55 in the placebo group were treated for symptomatic malaria at least once (per-protocol time to first infection vaccine efficacy 0·23, 95% CI –0·30 to 0·54, p=0·33; appendix 2 p 36). Median density of parasitaemia at first positive thick blood smear did not differ significantly by group (appendix 2 p 34). To assess efficacy against repeated *P falciparum* infections, we used a Poisson regression that models the number of positive thick blood smears as a function of time and treatment group. Vaccine efficacy (1–relative risk) was 31% (95% CI 1 to 51, p=0·0019) per protocol, and 29% (–1 to 50; p=0·026) by mITT.

By ELISA, automated immunofluorescence assay, and automated inhibition of sporozoite invasion assay, seroconversions were significantly more frequent and antibody levels were significantly higher in PfSPZ Vaccine recipients than controls (figure 3). Among PfSPZ Vaccine recipients, those who remained uninfected during follow up had significantly higher seroconversion rate, and significantly higher antibody levels, measured by ELISA and automated immunofluorescence assay, but not by automated inhibition of sporozoite invasion assay; antibody levels and seroconversions are described in more detail in appendix 2 (pp 16–17). Serum antibody responses by ELISA, automated immunofluorescence assay, and automated inhibition of sporozoite invasion assay are shown for individual participants (appendix 2 pp 37–45) and as ratios of post-immunisation to pre-immunisation values (appendix 2 pp 16–17, 46).

We assessed vaccine-induced T-cell responses using multiparameter flow cytometry on fresh whole blood.

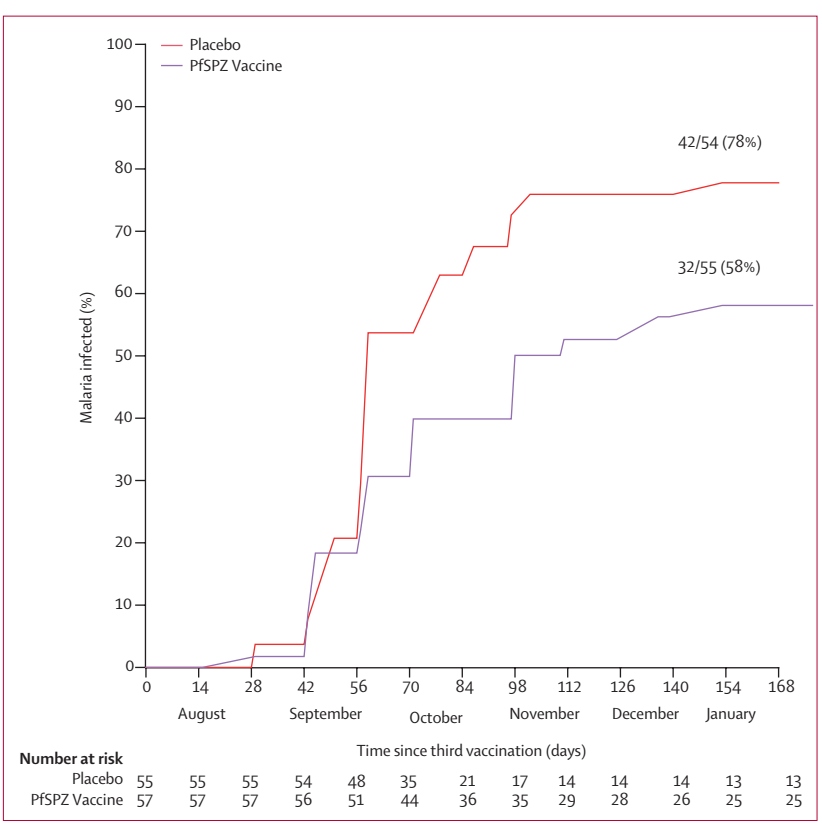


Figure 2: **Protective efficacy of PfSPZ Vaccine against naturally occurring infection**
Protective efficacy was analysed by time to first positive thick blood smear, with day 0 starting immediately after receipt of third dose of vaccination. The inverse survival curves include participants who received all three vaccinations and were evaluable for the primary efficacy endpoint (secondary trial outcome). PfSPZ=*Plasmodium falciparum* sporozoite.

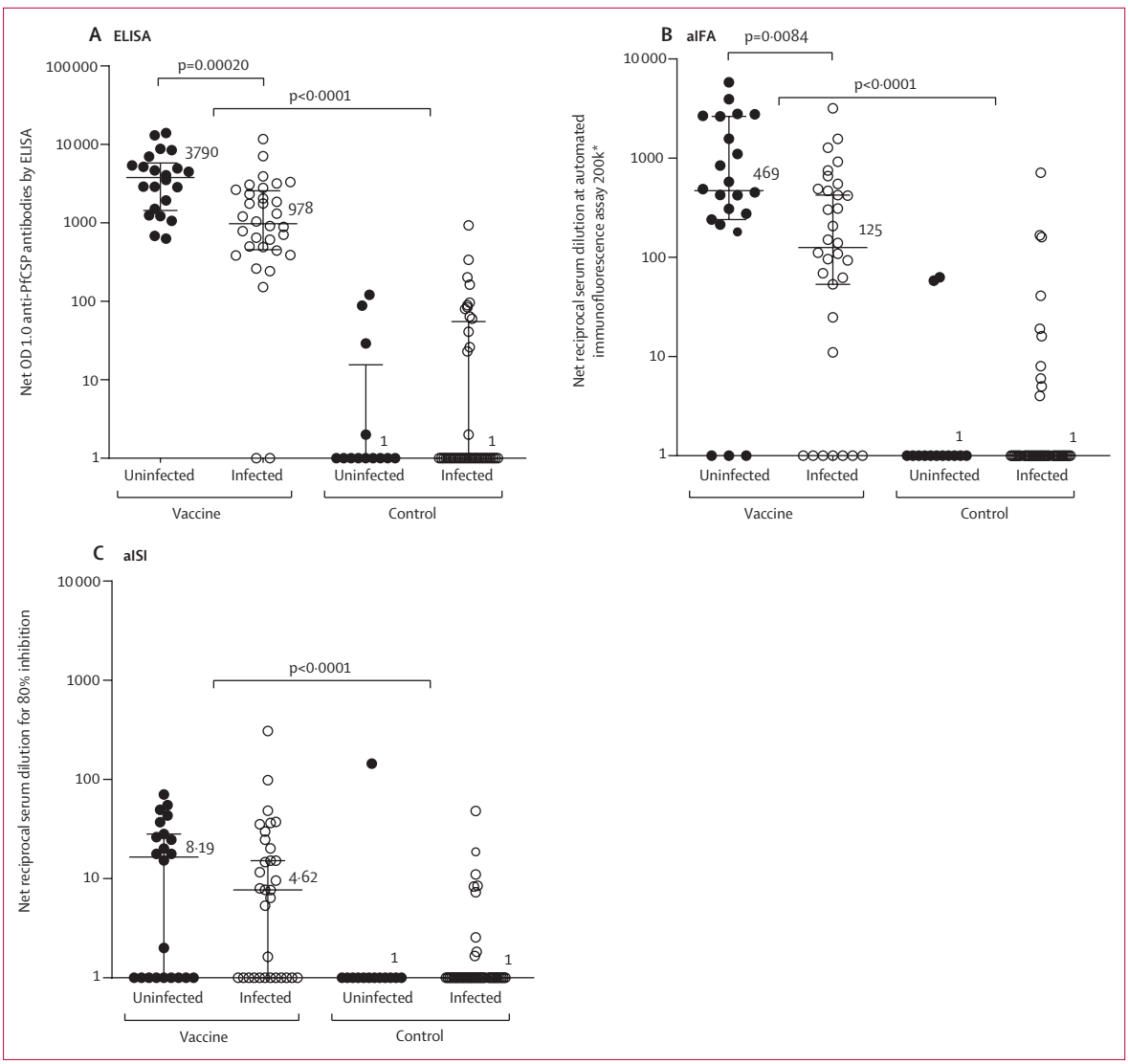


Figure 3: **Antibody responses measured after vaccination**
Antibody results for (A) PfCSP ELISA, (B) automated immunofluorescence assay, and (C) automated inhibition of sporozoite invasion assay. Net changes were obtained by subtracting pre-immune values from the values obtained from sera drawn 2 weeks after the third dose of $1{\cdot}8 \times 10^6$ PfSPZ. Analysis by Wilcoxon rank-sum test. PfSPZ=*Plasmodium falciparum* sporozoite. PfCSP=*P falciparum* circumsporozoite protein. OD=optical density. *Fluorescent intensity 2×10^5.

Ex-vivo measures were taken before and at 3, 7, 42, and 55 days after each vaccination, and every 4 weeks during follow-up (appendix 2 p 10). PfSPZ Vaccine and placebo recipients did not differ in their Vδ2 T-cell levels, measured as the percentage of total CD3 T cells (%Vδ2) at baseline (2·61% versus 2·43%; p=0·88, Wilcoxon rank-sum test); subsequent measures were calculated as fold change from baseline. Repeated measures were analysed by GEE, with a robust (sandwich) variance estimator applied to all models assuming autocorrelation structure (further details and GEE outputs in appendix 2 [pp 13–14, 47]).

During the immunisation period in the dry season, %Vδ2 decreased in placebo recipients but not PfSPZ Vaccine recipients (p=0·039, GEE; figure 4A). Among placebo recipients (figure 4B), %Vδ2 declined, and the decline was greater in uninfected than infected participants at the end of the dry season (day 120 median fold change 0·69 *vs* 0·83; p=0·015, GEE), and remained lower through the transmission season (day 281 median fold change 0·83 *vs* 0·99; p=0·008, GEE). Among PfSPZ Vaccine recipients (figure 4C), %Vδ2 increased during vaccination in uninfected (protected) but not infected (non-protected) vaccinees (day 120 median fold change 1·08 *vs* 0·99; p=0·045, GEE).

Overall, CD4 and CD8 T-cell values assessed as fold-change values from baseline did not differ between the placebo and PfSPZ Vaccine groups (appendix 2 p 49). T-cell responses are described in additional detail in appendix 2 (p 17).

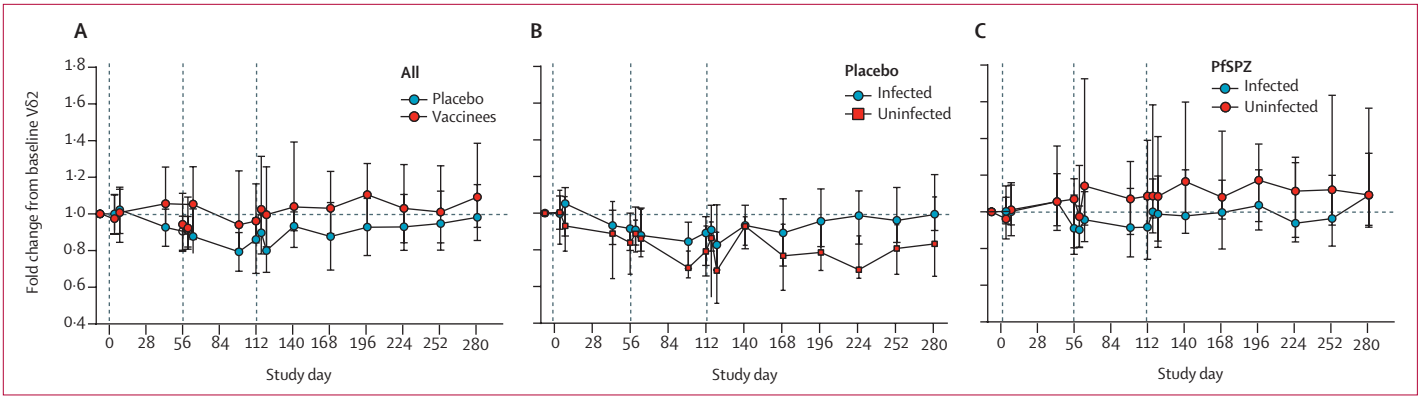


Figure 4: **Vδ2 γδ T-cell dynamics during vaccination and follow up**
Comparison of Vδ2 T cells in uninfected and infected vaccinees and controls. Fold change from baseline of Vδ2 T cells were compared between (A) PfSPZ and placebo groups, (B) uninfected and infected placebo volunteers, and (C) uninfected and infected PfSPZ Vaccine recipients. Data were analysed during the dry (vaccination) and the transmission (follow up) seasons using Wilcoxon rank-sum test at individual time points, and by generalised estimating equations for repeated measures. PfSPZ=*Plasmodium falciparum* sporozoite.

Discussion

We tested PfSPZ Vaccine for protection against intensely transmitted *P falciparum* in Malian adults with lifelong exposure to malaria. The aim of the trial was to evaluate whether a three-dose regimen would be safe, immunogenic, and efficacious in this population. To compensate for fewer doses than our previous study,[5] we administered four-fold higher cumulative dosage ($5·4\times10^6$ PfSPZ, compared with $1·35\times10^6$ PfSPZ). Our results showed that three doses were equally safe and well tolerated, induced high anti-PfCSP antibody responses, and conferred similar protection across a 24-week transmission season. These consistent findings give confidence that PfSPZ Vaccine is efficacious for protecting African adults from naturally occurring *P falciparum* infection and supports further PfSPZ Vaccine development and evaluation.

This is the first study to compare vaccine efficacy measured by CHMI with vaccine efficacy measured by natural exposure in the same population. In CHMI with parasites homologous to the vaccine strain (*P falciparum* NF54), vaccine efficacy 5 weeks after last vaccine dose was 100% measured by PCR, while in the main study, vaccine efficacy against natural infection measured by thick blood smear during 24 weeks was much lower. Furthermore, several pilot study participants also developed natural infections, despite sterile immunity against homologous CHMI and receipt of a fourth vaccine dose. Thus, vaccine efficacy measured by homologous CHMI at 5 weeks overestimated field vaccine efficacy over 24 weeks. The low rate of patent parasitaemia in Mali and other malaria-experienced populations during CHMI[17] contrasts with that in malaria-naive individuals, where PfSPZ Challenge (NF54) induces parasitaemia in 100% of participants.[13,18,19]

Despite the increased vaccine dosage in this trial, safety and reactogenicity remained excellent. As in our first trial, adverse event rates did not significantly differ from those in normal saline recipients. The safety record for PfSPZ Vaccine, including no evidence for product-associated fever, might warrant its testing in special populations such as pregnant women. The study also, unexpectedly, highlighted liver enzyme abnormalities in healthy adults after artesunate and amodiaquine, which could be underestimated if routine follow-up testing is not done. This finding is important, especially if pre-treatment is established as a component of the vaccine regimen, in which case safe and effective alternatives to artesunate and amodiaquine that optimise vaccine responses across multiple age groups and transmission settings require identification in future studies.

Our immunological studies identified non-mechanistic correlates of protection consistent with those we and others described in previous PfSPZ Vaccine trials.[10,20,21] Antibody levels against PfCSP and PfSPZ were significantly increased after the third dose of vaccine and were significantly higher in vaccine recipients who remained uninfected throughout the transmission season. However, the higher antibody responses observed in this trial versus our prior trial were not associated with an increased level of vaccine efficacy. Further, antibody levels in Malian vaccinees were substantially lower than those observed in unprotected US vaccinees (appendix 2 p 53), suggesting that PfCSP antibodies might not be a primary mediator of protection.[4] Consistent with our last study,[5] antibody responses after the same regimen in US participants were 9·3 times higher than in Malian participants (median optical density 1·0 of 16795 *vs* 1811; appendix 2 p 53),[22] possibly due to immune dysregulation. Antibody and T-cell responses to PfSPZ Vaccine are generally higher in older children than adults in Tanzania, raising the possibility that efficacy could prove higher in children than adults in Africa.[23]

In both US and Malian vaccinees, the Vδ2 subset of γδ T cells has been associated with protection against patent infection after PfSPZ Vaccine administration.[10,20,21] Here, we observed significant increases in Vδ2 T cells in vaccine recipients who remained uninfected throughout the transmission season. Although Vδ2 T cells are

associated with control of blood stage parasitaemia,[24,25] their expansion after PfSPZ vaccinations suggests a different role. In animal models of sporozoite vaccination, γδ T cells are required for induction of protective CD8 T cells, but are not required at infectious challenge to mediate sterile immunity.[20] Our inability to associate total peripheral CD8 T-cell levels to PfSPZ Vaccine administration is consistent with the observation that liver-resident CD8 T cells mediate activity.[4,10,26]

PfSPZ Vaccine is being developed for residents in endemic areas and for travellers to endemic areas.[6,27] The focus is Africa, where more than 98% of deaths and more than 90% of infections occur,[1] including most traveller infections. A practical regimen is crucial to clinical development plans for both target groups. Our study, in addition to confirming protection against intensely transmitted, heterogeneous *P falciparum* parasites in Mali, as in our previous five-dose study,[5] establishes that the same vaccine efficacy can be achieved with a practical three-dose regimen. This result led immediately to phase 2 assessments of three-dose regimens in Kenyan infants[28] and Gabonese children (NCT03521973), and laid the foundation for planned phase 3 trials of three-dose regimens in Equatoguinean adults and children and in malaria-naive US and European adults.

For the travellers' clinical development plans, both efficacy studies in Mali were paired with studies of the same five-dose and three-dose regimens in the USA.[22,29] with efficacy measured against CHMI using heterologous *P falciparum*. These comparisons establish heterologous CHMI as a rigorous test of vaccine efficacy in Africa, recognising that immune responses were five to 30 times lower in Malian than US participants.[5,29] These comparative studies have provided the foundation for a clinical development plan that will use heterologous CHMI for pivotal phase 3 trials in European and US participants. Studies in Africa are now directly comparing vaccine efficacy with 9×10^5 versus the $1{\cdot}8\times10^6$ PfSPZ dosing used here (NCT03989102).

This study had several limitations. Malaria varies widely by site in its presentation, transmission dynamics, response to control and treatment, and demographics of disease. Thus, any single study has potential limitations in reproducibility and generalisability. This was our second study at this site—same population, same season—hence we are confident in reproducibility, but caveat that generalisability remains limited and requires studies in other populations (perennial malaria transmission, less or more lifelong malaria exposure, children, and immunocompromised or pregnant individuals). We provided anti-malarial pre-treatment to avert potential suppression of vaccine responses, but other studies will be needed to confirm its usefulness. Another limitation is the length of follow-up: we followed up participants through one transmission season. We need to determine how long vaccine efficacy lasts and if booster doses are required. These are just some of the limitations that we are currently addressing in ongoing and planned clinical trials.

No other vaccine has previously been shown to prevent *P falciparum* infection in African adults across an entire malaria season of 24 weeks. The WHO recommended paediatric vaccine RTS,S/AS01E[2] did not confer significant efficacy in Kenyan adults against *P falciparum* infection during 14 weeks of follow-up,[3] although an earlier RTS,S formulation in a different adjuvant protected Gambian adults during the first 9 weeks but not the last 6 weeks of follow-up.[30] Current studies of PfSPZ Vaccine are exploring condensed three-dose regimens administered over 4 weeks for flexibility in mass vaccination programmes and for special populations such as pregnant women. PfSPZ Vaccine must be assessed in combination with other interventions to pursue elimination. Finally, the efficacy of boosting should be assessed, as giving a single booster dose following primary vaccination will simplify efforts to use PfSPZ Vaccine on a seasonal basis.

Contributors

MSS, SAH, and PED were the principal investigators. MSS, SAH, IZ, TLR, SLH, OD, and PED designed the trial with contributions from all authors in review of the approved final version and additional amendments. MSS, SAH, AK, IZ, BK, YS, KS, AM, JL, AI, RM, IT, COG, AD, KN, FK, AN, AZ, MAG, and NK collected and cleaned the data. MSS, SAH, AK, BK, AI, RM, SLH, and PED accessed and verified the data. IZ, COG, FK, AZ, MAG, AB, and NK completed the study laboratory endpoints. YA, ERJ, BKLS, PFB, and SLH developed the vaccine. AD, KN, AN, KR, AZ, YA, ERJ, and BKLS completed procedures related to vaccinations. TM provided regulatory support during the course of the study. IZ, ZH, and NK completed the statistical analysis. MSS, SAH, IZ, ZH, NK, SLH, OD, and PED interpreted data and results. All authors had full access to all study data and had final responsibility for the decision to submit for publication.

Declaration of interests

YA, ERJ, TM, NK, BKLS, PFB, TLR, and SLH are salaried, full-time employees of Sanaria, the developer and sponsor of Sanaria PfSPZ Vaccine. All other authors declare no competing interests.

Data sharing

Individual-level participant data will be made available with publication and upon execution of inter-institutional human data sharing agreement. Data can include all those described in the manuscript.

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